Remarks

The Office Action mailed May 31, 2006 has been carefully reviewed and the foregoing remarks are submitted in consequence thereof.

In accordance with 37 C.F.R. 1.136(a), a one-month extension of time is submitted herewith to extend the due date of the response to the Office Action dated May 31, 2006, for the above identified patent application from August 31, 2006, through and including October 2, 2006 (due to September 30 falling on a Saturday). In accordance with 37 C.F.R. 1.17(a)(1), authorization to charge a deposit account in the amount of $60.00 to cover this extension of time request also is submitted herewith.

Claims 1-12 are rejected. Claims 2-12 are objected to. Claims 13-18 have been withdrawn from consideration. Pursuant to this Amendment, Claims 1-12 have been canceled. Claims 19-30 have been newly added. No additional fee is due for newly added Claims 19-30. Claims 13-30 are pending.

The objection to the disclosure is respectfully traversed. Applicants have amended paragraph [00257]. No new matter has been added.

For the reasons set forth above, Applicants request that the objection to the disclosure be withdrawn.

The objections to Claims 2-12 due to informalities are respectfully traversed. Applicants have canceled Claims 2-12 and added Claims 19-30. Thus, the objections to Claims 2-12 are moot.

For the reasons set forth above, Applicants request that the objections to Claims 2-12 be withdrawn.

The rejection of Claims 1-9, 11, and 12 under 35 U.S.C. § 112, first paragraph, for failing to enable one skilled in the art to make and/or use the invention commensurate in scope with the claims is respectfully traversed.

Applicants traverse this rejection. Claims 1-12 have been canceled. Accordingly, Applicants respectfully request that the Section 112, first paragraph, rejection of Claims 1-9, 11, and 12 be withdrawn.

Furthermore, Applicants respectfully submit that newly added Claims 19-30 satisfy the enablement requirement of Section 112, first paragraph.

Using the multi-factor test found in *In re Wands*, the Office Action found the specification non-enabling with respect to the use of a non-fish promoter (as found in canceled Claims 1-9, 11, and 12) and the use of promoters specific to skeletal, heart, or cartilage-specific expression (as found in canceled Claims 7-9). The Office Action states that "the art has established an unpredictability with respect to the activity of non-fish promoters in transgenic fish...[and]...it would require undue experimentation to determine which promoters encompassed by the claims, other than fish promoters, could be effectively used in the claimed invention." Applicants traverse this assertion and respectfully submit the following for Examiner's consideration:

The state of art at the time of filing as well as the relative skill of those in the art show that undue experimentation is not required to make and use the invention. First, due to functional conservation of regulatory DNA sequences across species, non-fish promoter/enhancer elements are sufficient to direct transgene expression in appropriate spatially and temporally restricted patterns in transgenic zebrafish. This is supported by several references, which are submitted with an accompanying Information Disclosure Statement. For example:

- Westerfield et al., 1992, Genes Dev. 6: 591-98 – teaches the use of mouse Hox-1.1 and human Hox-3.3 promoters to drive region and tissue specific transgene expression in transgenic zebrafish. (Abstract submitted with the accompanying Information Disclosure Statement.)

- Reinhard et al., 1994, Development 120: 1767-75 – teaches the use of a rat Gap43 promoter to drive expression of a β-galactosidase reporter specifically in neurons – and further characterization of functionally conserved DNA sequences - of transgenic zebrafish.

- Moss et al., 1996, Gene 173: 89-98 – teaches the use of rat myosin light-chain enhancer regions to drive expression of a GFP reporter specifically in skeletal muscle cells of transgenic zebrafish.

- Motoike et al., 2000, Genesis 28: 75-81 – teaches the use of mouse Tie2 promoters to drive expression of a GFP reporter specifically in vascular endothelial cells of transgenic zebrafish.

- Udvadia et al., 2001, Development 128: 1175-1182 – teaches the use a rat Gap43 promoter to drive expression of a GFP reporter specifically in neurons of transgenic zebrafish.

- Perkins et al., 2002, Visual Neurosci. 19: 257-264 – teaches the use of a frog opsin promoter to drive expression of a GFP reporter specifically in rod photoreceptors of the retina of transgenic zebrafish.

Second, other references demonstrate that the relative skill of those in the art could practice and make the invention with the amount of direction and guidance presented in the specification. For example, Applicants were successfully practicing the art in question, at the time the application was filed, toward the derivation of transgenic zebrafish lines expressing fluorescent reporters within specific subsets of cells within the eye (Kay et al., 2004, Develop, 131: 1331-1342; Godinho et al., 2005, Develop, 132: 5069-5079). Specifically, *Pax4DF4:MGFP*, *Pax4DF4:MCFP*, and *Pax4DF4:MYFP* transgenic lines were created using *Xenopus*-derived *ef1α* promoter and *Coturnix* (quail)-derived *pax6* enhancer elements, to direct expression of membrane-targeted fluorescent reporter proteins in isolated subsets of amacrine interneurons in the zebrafish retina. This expression pattern is, moreover, evolutionarily conserved with respect to the *pax6* enhancer elements.

Third, scientific advances – most notably the sequencing of human, mouse, zebrafish, etc., genomes, computational methods for automated cross-species sequence comparisons, the creation of publicly accessible gene expression databases, the advent of bacterial recombination within artificial chromosome systems, and the use of fluorescent reporters – facilitate the approach of using either homologous or heterologous promoters, and associated regulatory DNA sequences, for transgenesis in zebrafish via procedurally identical methodologies. As the Examiner finds in the Office Action, the specification provides an

exemplary teaching of the art in question (i.e. the process of selecting, isolating, amplifying, and/or integrating candidate regulatory DNA sequences such that they are operably linked to the transgene product) in the section involving the use of the pufferfish (Fugu) ChAT locus to derive transgenic zebrafish lines with expression specifically in ChAT-expressing neurons (paragraph [000249] and [000262]).

Moreover, the experimentation required to achieve tissue- or cell-specific transgenic expression patterns in fish, particularly with regard to in vivo expression assays of transgenic zebrafish, cannot be deemed "undue experimentation." The experimentation required only involves routine screening, regardless of the origin of the regulatory DNA sequences being utilized. Specifically, the art of identifying a genetic locus of interest regarding a specific expression pattern (i.e., isolating, amplifying, and/or integrating corresponding candidate regulatory sequences such that they are operably linked to the transgene of the invention, and testing the transgenic construct via transient transgenesis in zebrafish) is a procedurally identical methodology regardless of the species origin of the regulatory DNA sequences being screened. Furthermore, unique advantages of the zebrafish system allow DNA regulatory elements to be screened via transient transgenesis assays that reduce the process to one of routine screening (Rothenberg, 2001, PNAS 98: 6540-6542). As this reference states "[b]y injecting pufferfish genomic cosmids into zebrafish zygotes and testing their expression in the resulting embryos by in situ hybridization, a rapid determination can be made of the location of sequences controlling expression in each of several embryonic domains at once (11). Thus, noncoding DNA regions can be scanned relatively easily for the sequences that are necessary or sufficient to drive a wide range of tissue specific expression patterns." (page 6540, column 3, paragraph 1).

Accordingly, the specification enables one skilled in the art to make and/or use the invention commensurate in scope with the claims. As the Examiner acknowledged in the Office Action, the use of homologous regulatory DNA sequences are enabled by the specification. The specification also teaches the use of transgenic zebrafish for screening DNA regulatory elements that are sufficient for specific expression patterns. Furthermore, published references support the use of heterologous promoters in fish. Lastly, the process of screening DNA sequences for those that confer tissue and cell type specific expression in transgenic zebrafish a) is independent of the origin of the regulatory sequence being screened

and b) involves a routine screening process that the specification and published references teach.

Thus, Applicants respectfully submit that the specification is enabling with respect to newly added Claims 19-30.

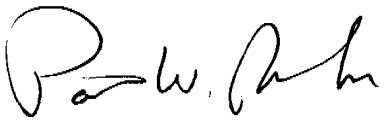
The rejection of Claims 4-11 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention is respectfully traversed.

The Office Action asserts that there is insufficient antecedent basis for "the regulatory DNA sequence." Applicants have canceled Claims 4-11. Furthermore, it is respectfully submitted that newly added Claims 19-30 have sufficient antecedent basis for "the regulatory DNA sequence."

For the reasons set forth above, Applicants request that the Section 112, second paragraph, rejection to Claims 4-11 be withdrawn.

In view of the foregoing remarks, all the claims in this application are believed to be in condition for allowance. Reconsideration and favorable action is respectfully solicited.

Respectfully Submitted,



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